REMARKS

The Official Action dated November 6, 2002 and the references cited therein have been carefully reviewed. In view of the amendments presented herewith and the following remarks, favorable reconsideration and allowance of this application are respectfully requested.

Status of the prosecution:

Claims 1 and 3-9 are pending in this application. The November 6, 2002 Official Action is a second, non-final action on the merits. All pending claims remain rejected or stand newly rejected, and certain objections to the specification and claims have been made.

The specification was objected to as containing an embedded hyperlink. Claim 1 was objected to for recitation of "a genome." The examiner suggested amending the claim to recite: "the genome."

Claims 3, 4, 7 and 9 remain rejected or stand newly rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness. In claim 3, the examiner stated that the sole designation of a marker by a non-art recognized designation creates ambiguity, and suggests amending the claim to identify the claimed marker by SEQ ID number. Claim 4 is alleged to be indefinite due to lack of clarity as to which marker each SEQ ID number is directed in the list of species in claim 3. Claim 7 is allegedly indefinite in the phrase "the gene." Claim 8 and dependent claim 9 are deemed unclear as being generally narrative. The examiner suggested amending the claim to recite "an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:5, or a nucleotide sequence complementary thereto."

Claims 1 and 3-7 stand newly rejected under 35 U.S.C. §112, first paragraph, for alleged lack of adequate written description in the specification. This rejection is based on the assertion that the specification does not describe (1) the composition or structure of the late blight resistance gene on chromosome 8 of *S. bulbocastanum*; (2) late blight-resistant potato plants having incorporated the relevant portion of *S. bulbocastanum* chromosome 8 by traditional breeding methods; and (3) RFLP markers CT148, CT252 and CT68. Therefore, the specification is deemed not to adequately describe the invention as claimed.

Claims 1 and 3-7 also stand newly rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement. According to the examiner, the specification is enabling for the claimed potato plants produced by somatic hybridization between *S. tuberosum* and *S. bulbocastanum*, but it does not enable the production of any potato plant comprising a segment of *S. bulbocastanum* chromosome 8 having a gene that confers late blight resistance. At page 10 of the Action, the examiner appears to indicate that a deposit of the claimed plant, seeds or regenerable plant tissue would overcome this rejection.

Claims 1 and 6 remain rejected, and claims 3 and 4 stand newly rejected under 35 U.S.C. §102(b) as allegedly anticipated by Schumman et al. (Phys. Plantarum 82: A23 (Abstract 134), 1990) taken with the evidence of Naess et al. (Theor. Appl. Genet. 101: 697-704, 2000). The examiner stated that (1) Schumann et al. disclose a somatic hybrid potato plant produced by fusion of protoplasts isolated from *S. tuberosum* and *S. bulbocastanum* and exhibiting improved resistance against late blight; and (2) Naess et al. disclose that resistance in such a somatic hybrid potato is inherently associated with *S. bulbocastanum* chromosome 8 and RFLP marker CT88 (SEQ ID NO:3). Schumann et al. was therefore deemed to inherently disclose all of the limitations of the rejected claims.

Claims 7-9 were deemed free of the prior art.

Amendments presented in this paper:

In accordance with the present amendment, the specification has been amended to remove the embedded hyperlink at pages 11 and 22 of the specification. The specification was also amended to insert a fuller description of the priority information for the application.

Claim 3 was canceled and claims 1, 4, 7 and 8 were amended. Claim 1 was amended to incorporate the limitations previously recited in claim 3. Claim 4 was amended to specify sequence identification numbers associated with certain of the markers now recited in claim 1. Claim 7 was amended to recite the segment of *S. bulbocastanum* chromosome 8 as recited in amended claim 1. Claim 8 was amended to call for an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:5, or a nucleotide sequence complementary thereto.

New claims 16-19 were added. Claim 16 is directed to a late blight-resistant potato plant that is a backcross progeny of a somatic hybrid of *Solanum tuberosum* and *Solanum bulbocastanum*, wherein the plant comprises a segment of chromosome 8 of the *Solanum bulbocastanum* genome having a gene that confers the late blight resistance. Support for new claim 16 is found throughout the specification. New claims 17-19 depend from claim 16 and are drawn to subject matter that parallels that of claims 3, 4 and 5. No new matter has been added by the claim amendments or new claims.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "Version with markings to show changes made."

Applicants assert that the foregoing specification and claim amendments overcome each of the objections and rejections issued in the November 6, 2002 Official Action, and that

the claims as amended and the new claims are in condition for allowance. Support for Applicants' assertion to this effect is set forth below.

Objection to the specification and claims:

The specification was objected to for containing embedded hyperlinks. The embedded hyperlinks have been removed, hence this objection should be overcome and its withdrawal is requested.

Claim 1 was objected to for recitation of "a genome." In accordance with the examiner's suggestion, claim 1 has been re-written to recite "the genome." Accordingly, withdrawal of this objection is requested.

Rejections under 35 U.S.C. §112, second paragraph:

Claims 3, 4, 7 and 9 remain rejected or stand newly rejected under 35 U.S.C. §112, second paragraph. In claim 3, the examiner stated that the sole designation of a marker by a non-art recognized designation creates ambiguity and renders the metes and bounds of the claim indeterminable. Claim 3 has been canceled; however, Applicants respectfully traverse this rejection as applied to claim 1, which now includes the limitations of canceled claim 3. Designation of genetic markers as RFLP or RAPD markers in connection with a specified genome is art-recognized. It is customary for geneticists studying a particular genome to pool mapping information in a central location, thereby enriching the knowledge base for all. Examples of such pooled databases are found throughout the literature. Of particular relevance in the present invention is the Solanaceae Genomics Network (www.sgn.cornell.edu), which exhibits detailed map information on genomes of solanaceaous plants (including potato) and enables one to identify a RFLP sequence associated therewith

simply by typing in the name of the marker. Printed pages of the website are attached to this response as Exhibit 1. To illustrate, the undersigned attorney entered each of the RFLP markers recited in claim 3 into the query box of the website – these were CT88, CT148, CT252 and CT68. This resulted in identification not only of a list genomes in which the marker had been found, but in a reference sequence as well. From this example, it is clear that persons of skill in the art use RFLP designations that correspond to particular sequences (which will be slightly varied as described in the specification for CT88); thus these are not arbitrary designations. RAPD markers are used similarly, as evidenced by, e.g. Naess et al. (2000) Theor. Appl. Genet. 101: 697-704 (cited by the examiner for a different purpose) and an excerpt from the USDA website attached hereto as Exhibit 2, entitled "USDA Plant Genome Research Progress Report (see page 3, first full paragraph). The present specification gives examples of sequences associated with the RAPD markers recited in claim 1, and further discloses where primers for other RAPD markers may be obtained (page 10, lines 12-13). From this information, a person of skill in the art would be able to identify the RAPD markers as designated in claim 1. For these reasons, the designations are not arbitrary and the metes and bounds of claim 1 are clear to the skilled artisan. Accordingly, withdrawal of the indefiniteness rejection is requested.

Claim 4 is alleged to be indefinite due to lack of clarity as to which marker each SEQ ID number is directed in the list of species in claim 3. Claim 4 has been amended such that the SEQ ID numbers associated with particular RAPD or RFLP markers are clear. Accordingly, the indefiniteness rejection of this claim should be overcome, and its withdrawal is requested.

Claim 7 is allegedly indefinite in the phrase "the gene." Claim 7 has been amended such that this phrase is no longer recited. Applicants submit that claim 7 as amended clearly

points out and distinctly claims the subject matter regarded as the invention. Accordingly, withdrawal of the rejection under 35 U.S.C. §112, second paragraph, is requested.

Claim 8 and dependent claim 9 are deemed unclear as being generally narrative. Claim 8 has been amended in accordance with the examiner's suggestion to recite "an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:5, or a nucleotide sequence complementary thereto." Withdrawal of the rejection is therefore requested.

Rejection under 35 U.S.C. §112, first paragraph (written description):

Claims 1 and 3-7 stand rejected under 35 U.S.C. §112, first paragraph, for alleged lack of adequate written description in the specification. This rejection is based on the assertion that the specification does not describe (1) the composition or structure of the late blight resistance gene on chromosome 8 of *S. bulbocastanum*; (2) late blight-resistant potato plants having incorporated the relevant portion of *S. bulbocastanum* chromosome 8 by traditional breeding methods; and (3) RFLP markers CT148, CT252 and CT68. Therefore, the specification is deemed not to adequately describe the invention as claimed. Applicants traverse this rejection as applied to the presently amended claims, as well as the new claims.

The adequacy of a written description is a question of fact that must be determined on a case-by case basis. MPEP §2163. A written description is given a strong presumption of adequacy and rejection of original claims for lack of written description should be rare. *Id.* An examiner must overcome the presumption of adequacy by putting forth, on a reasonable basis, sufficient evidence or reasoning. *In re Wertheim*, 541 F.2d 257, 263 (CCPA 1976). Arguing lack of literal support is not enough since the invention need not be described in *ipsis verbis* to satisfy the written description requirement. *Id.* at 265.

As the Federal Circuit has stated: ". . .the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed*." *Vas-Cath Inc. et al. v. Mahurkar et al.,* 935 F.2d 1555, 1563-4 (Fed. Cir. 1991) (emphasis in original). A preponderance of evidence is required as to why a skilled artisan would not recognize a description of the claimed invention, as that is the perspective from which satisfaction of the requirement is measured. *Amgen Inc. v. Hoechst Marion Roussel, Inc. et al.,* No. 01-1191, 01-1218, 2003 U.S. App. LEXIS 118 at *35 (Fed. Cir. 2003) citing *Lockwood v. Am. Airlines, Inc.,* 107 F.3d 1565, 1572 (Fed. Cir. 1997); see also MPEP §2163.

Possession of the invention may be established through words, structures, figures, diagrams and formulas that fully set forth the claimed invention. *Lockwood*, 107 F.3d at 1572. "Generally there is an inverse correlation between the level of skill and knowledge in the art and the specificity of the disclosure necessary to satisfy the written description requirement." MPEP §2163.

In the instant specification, the Applicants have disclosed the mapping of one genetic source of late blight resistance in *S. bulbocastanum* to chromosome 8, and have linked it to at least two RAPD markers and five RFLP markers, the physical sequences of which are either disclosed in the specification or are readily available in the literature (the latter being the case for RFLPS CT148, CT252 and CT68). The specification further discloses the production of somatic hybrids of *S. tuberosum* and *S. bulbocastanum* and numerous backcross progeny thereof, wherein the presence of late blight resistance is at least 95% correlated with the presence of one or more of the RAPD or RFLP markers. Claims 1 and 3-7 as amended are drawn to a late blight-resistant potato plant comprising a segment of chromosome 8 of the

genome from *Solanum bulbocastanum* which comprises a gene that confers the resistance to late blight, wherein the gene conferring the late blight resistance co-segregates with a marker selected from the group consisting of a $GO2_{586}$ RAPD marker, a $PO9_{587}$ RAPD marker, a CT88 RFLP marker, a CT148 RFLP marker, a CT252 RFLP marker and a CT68 RFLP marker, associated sequences of which are either disclosed in the specification or available in the literature. Accordingly, the skilled artisan can envision the claimed structure (i.e., the potato plant) in accordance with the specification and would understand that the inventors were in possession of the claimed invention at the time of filing. With respect to the examiner's concern that the composition or structure of the resistance-conferring segment of *S. bulbocastanum* chromosome 8 is not described, it is respectfully asserted that claims 1 and 3-7 do not claim that segment as a separate entity, but instead claim a potato plant comprising that element. One need not know the composition (sequence) of a DNA segment in order to isolate it and introduce it into other organisms. In view of the genetic and physical markers associated with the resistance-conferring segment of *S. bulbocastanum* chromosome 8 disclosed in the specification and recited in claim 1, one of skill in the art would reasonably find that the inventors were in possession of more than enough information to isolate the segment from *S. bulbocastanum* and introduce it into *S. tuberosum*.

Thus, in accordance with the holding in *Amgen*, sufficient information has been conveyed in the present specification such that those of skill in the art would recognize the description of the late blight-resistant potato plants as claimed. The specification conveys, to those of skill in the art, distinguishing information concerning the identity of the plants such that one of skill could visualize or recognize the identity of the members of the genus of claimed plants. In particular, the specification discloses functional information, as well as structural information required for the skilled artisan to correlate the function with known

structures – i.e., the presence of the relevant portion of *S. bulbocastanum* chromosome 8 as evidenced by one or more linked RAPD or RFLP markers. In view of the foregoing, Applicants respectfully request the withdrawal of the rejection for lack of adequate written description under 35 U.S.C. §112.

With respect to new claims 16-19, Applicants note that the examiner has already acknowledged that the specification provides an adequate written description supporting claims drawn to somatic hybrids of *S. tuberosum* and *S. bulbocastanum* and, presumably, to their progeny, the production of which is described in the specification. Accordingly, the rejection for lack of adequate written description should not be applied to new claims 16-19.

Rejection under 35 U.S.C. §112, first paragraph (enablement):

Claims 1 and 3-7 also stand rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement. According to the examiner, the specification is enabling for the claimed potato plants produced by somatic hybridization between *S. tuberosum* and *S. bulbocastanum*, but it does not enable the production of any potato plant comprising a segment of *S. bulbocastanum* chromosome 8 having a gene that confers late blight resistance. At page 10 of the Action, the examiner appears to indicate that a deposit of the claimed plant, seeds or regenerable plant tissue would overcome this rejection.

Applicants traverse this rejection as applied to the presently amended claims and to the new claims, and further assert that a deposit of the claimed plant is not needed. Claim 1 as amended is drawn to a late blight-resistant potato plant comprising a segment of chromosome 8 of the genome from *Solanum bulbocastanum* which comprises a gene that confers said resistance to late blight, wherein the gene conferring the late blight resistance co-segregates with a marker selected from the group consisting of a $GO2_{586}$ RAPD marker, a

$PO9_{587}$ RAPD marker, a CT88 RFLP marker, a CT148 RFLP marker, a CT252 RFLP marker and a CT68 RFLP marker, associated sequences of which are either disclosed in the specification or available in the literature

The question of enablement is a question of law, based on underlying factual determination. *Amgen, Inc. v. Hoechst Marion Roussel, Inc. et al.*, No. 01-1191, 01-1218, 2003 U.S. App. LEXIS 118 at *48 (Fed. Cir. 2003). Before any analysis of enablement can occur, it is necessary for the examiner to construe the claims. The examiner should always look for enabled, allowable subject matter and communicate to applicants what that subject matter is at the earliest point possible in the prosecution of the application. (MPEP 2164.04).

In the instant case, the examiner has set out claimed subject matter deemed enabled by the specification, namely, potato plants produced by somatic hybridization between *S. tuberosum* and *S. bulbocastanum*. However, the examiner has deemed the specification not to enable either (1) transgenic potato plants or (2) plants produced by conventional breeding techniques, which contain the relevant portion of *S. bulbocastanum* chromosome 8. In view of the teachings of the specification and the level of skill in the art of plant genetics and molecular biology, Applicants respectfully disagree with the examiner and assert that claim 1 as amended and claims dependent therefrom are fully enabled.

The Federal Circuit has consistently held that "the specification must teach those of ordinary skill in the art how to make and use the full scope of **the invention** without undue experimentation. *In re Wright*, 999 F.2d 1557,1561(Fed. Cir. 1993). The fact that a quantity of experimentation, even complex experimentation, may be required is not dispositive of the analysis (MPEP 2164.04). The key word is "undue," not "experimentation". *Angstadt*, 537 F.2d at 504.

Not everything necessary to practice the invention need be disclosed. The Federal

Circuit has stated that what is well-known is best omitted. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991). Further, the scope of enablement must only bear a reasonable connection to the scope of the claims. *See, e.g., In re Fisher*, 427 F.2d 833, 839 (CCPA 1970). Additionally, as the Federal Circuit recently reiterated, the law is clear that the specification need teach only one mode of making and using a claimed invention. *Amgen*, 2003 U.S. App. LEXIS 118 at *50.

In the present case, the specification teaches a late blight-resistant potato plant produced by a combination of (1) somatic hybridization between *S. tuberosum* and *S. bulbocastanum* and (2) traditional breeding methods comprising backcrossing with *S. tuberosum* and selecting progeny having disease resistance co-segregating with a physical marker comprising a RFLP or RAPD fragment sequence associated with the relevant portion of *S. bulbocastanum* chromosome 8. Thus, the specification has met the enablement requirement of teaching a mode of making and using the invention as claimed in claim 1. *Amgen*, 2003 U.S. App. LEXIS 118 at *50. Arguably, the inquiry into enablement should end here. However, Applicants respectfully assert further that one skilled in the art would also be able to produce the potato plant of claim 1 by recombinant means, in view of the recitation and teachings of physical markers associated with the late blight resistance-conferring segment of *S. bulbocastanum* chromosome 8, and the level of skill in the pertinent art. Using these physical markers set forth in the specification, it would be well within the purview of the skilled artisan to isolate a segment from *S. bulbocastanum* chromosome 8 by hybridization to one of the disclosed or known RFLP or RAPD sequences, to introduce such a segment into *S. tuberosum* cells, and to test transgenic plants thereby produced for late blight resistance. The fact that a quantity of experimentation, even complex experimentation, may be required is not dispositive of the analysis (MPEP §2164.04). In view of the high

level of skill in the pertinent art, the experimentation required to accomplish this task is not undue.

For the foregoing reasons, Applicants respectfully assert that claims 1 and 4-7 are fully enabled by the specification. Accordingly, withdrawal of their rejection for lack of enablement is requested.

Turning next to new claims 16-19, Applicants note as stated above that the examiner has already acknowledged that the specification enables claims drawn to somatic hybrids of *S. tuberosum* and *S. bulbocastanum* and, presumably, to their progeny, the production of which is described in the specification. Accordingly, the rejection for lack of enablement should not be applied to new claims 16-19.

Rejection under 35 U.S.C. §102(b):

Claims 1, 3, 4 and 6 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Schumann et al., disclosing a somatic hybrid potato plant produced by fusion of protoplasts isolated from *S. tuberosum* and *S. bulbocastanum* and exhibiting improved resistance against late blight, in light of the evidence set forth by Naess et al., disclosing that resistance in a *S. tuberosum – S. bulbocastanum* somatic hybrid potato is associated with *S. bulbocastanum* chromosome 8 and RFLP marker CT88 (SEQ ID NO:3). Based on Naess et al., Schumann et al. was deemed to inherently disclose all of the limitations of the rejected claims.

Applicants traverse this rejection. If a prior art rejection relies on the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to support the determination that the inherent characteristic necessarily flows from the teachings of the applied prior art. MPEP §2112, citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App.

& Inter. 1990 (emphasis in original). In the instant case, the mere fact that the late blight-resistance in the hybrids disclosed by Naess et al. was traced to *S. bulbocastanum* chromosome 8 does not necessarily mean that the late blight resistance in the hybrids of Schumann et al. was conferred by any portion of *S. bulbocastanum* chromosome 8. Indeed, late blight resistance in wild potato species has been associated with many different resistance genes. For instance, *S. demissum* contains at least eleven such genes, dispersed across at least three chromosomes (see Exhibit 3). In *S. pinnatisectum*, a late blight resistance gene is found on chromosome 7 (see Exhibit 4). The wild potato *S. verrucosum* displays vertical and horizontal late blight resistance (see Exhibit 5), wherein the race-specific resistance patterns reflect a model of complementary action of two or more genes. Considering this information, how can it be said that the resistance observed in the hybrids of Schumann et al. necessarily arises from *S. bulbocastanum* chromosome 8? It cannot be said, in view of the existence of probability that *S. bulbocastanum*, like other wild potato species, contains more than one gene for resistance to late blight, which may be distributed among several chromosomes. The fact that a certain characteristic may be present in the prior art is not sufficient to establish the inherency of that result or characteristic. MPEP §2112, citing *In re Rijckaert*, 9 F.3d, 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). Therefore, Schumann et al. cannot be deemed to inherently disclose the claimed invention merely because the hybrids disclosed therein may contain a segment of *S. bulbocastanum* chromosome 8 that confers the reported late blight resistance. Accordingly, the rejection under 35 U.S.C. §102(b) is untenable, and should be withdrawn.

Conclusion:

In view of the amendments submitted herewith and the foregoing remarks, the presently pending claims are believed to be in condition for allowance. Applicants respectfully request early and favorable reconsideration and withdrawal of the objections and rejections set forth in the November 6, 2002 Official Action, and allowance of this application.

Respectfully submitted,

Date: March 4, 2003

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The specification was amended as follows:

The first paragraph on page 1 of the specification was amended as shown below.

--This application is a national stage application under 35 U.S.C. §371 of International Application PCT/US98/15910, filed July 27, 1998, which claims benefit of [claims priority to] U.S. Provisional Application Serial No. 60/054,267, filed July 30, 1997, which is incorporated by reference herein in its entirety.--

The paragraph beginning on page 10, line 34 and ending on page 11, line 8, was amended as shown below.

--This invention further provides RFLP molecular markers, useful in facilitating selection of breeding progeny that contain the resistance-conferring segment of *S. bulbocastanum*. These markers can also assist in defining the location of the resistance genes on the chromosome, and obtaining isolated genomic segments containing the gene(s). The nucleotide sequence of RFLP CT88 from three different sources (published by Tanksley et al. ("Solgenes" database, United States Department of Agriculture, National Agricultural Library (NAL) Probe Newsletter) [(http://probe.nalusda.gov:8300/cgi-bin/browse/solgenes)], from R4 potato, and from *S. bulbocastanum*) are set forth herein as SEQ ID NOS: 3, 4 and 5 respectively.--

The paragraph beginning on page 22, line 35 and ending on page 23, line 3, was amended as shown below.

--The nucleotide sequence of $GO2_{586}$ is set forth herein as SEQ ID NO: 1. The nucleotide sequence of $PO9_{587}$ is set forth herein as SEQ ID NO:2. Three nucleotide sequences of CT88 are set forth herein. SEQ ID NO:3 is the sequence published by Tanksley et al. ("Solgenes" database, United States Department of Agriculture, National Agricultural Library (NAL) Probe Newsletter) [http://probe.nalusda.gov.8300/cgi-bin/browse/solgenes)]; SEQ ID NO:4 is from R4 potato, and SEQ ID NO:5 is from *S. bulbocastanum* (PT29). Slight differences were noted among the three sequences. The R4 potato marker is 589 bp in length, while the *S. bulbocastanum* RFLP is 592 bp and the Tanksley et al. sequence is 596 bp. In addition, the *S. bulbocastanum* CT88 homolog possesses two TaqI sites, whereas the other two have only one.--

The claims were amended as follows:

Claim 3 was canceled.

Claims 1, 4, 7 and 8 were amended as shown below.

1. (Amended) A late blight-resistant potato plant comprising a segment of chromosome 8 of the [a] genome from *Solanum bulbocastanum* which comprises a gene that confers said resistance to late blight, wherein the gene conferring the late blight resistance co-segregates with a marker selected from the group consisting of a $GO2_{586}$ RAPD marker, a $PO9_{587}$ RAPD marker, a CT88 RFLP marker, a CT148 RFLP marker, a CT252 RFLP marker and a CT68 RFLP marker.

4. (Twice amended) The potato plant of claim 1 [3] wherein the $GO2_{586}$ RAPD marker comprises [a sequence selected from the group consisting of] SEQ ID NO:1, the $PO9_{587}$ RAPD marker comprises SEQ ID NO:2, and the CT88 RFLP marker comprises SEQ ID NO:3, SEQ ID NO:4 or [and] SEQ ID NO:5.

7. (Amended) The potato plant of claim 1, wherein the segment of chromosome 8 of the genome from *Solanum bulbocastanum* which comprises the gene that confers the resistance to late blight [resistance gene] is incorporated into the plant by genetic transformation of a cell of the plant with a plant transforming vector comprising the segment [gene].

8. (Twice amended) An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:5, or a nucleotide sequence complementary thereto [which is complementary to either strand of a double-stranded DNA molecule, wherein one strand of the DNA molecule comprises SEQ ID NO:5].

New claims 16-19 were added, as set forth below.

16. (New) A late blight-resistant potato plant that is a backcross progeny of a somatic hybrid of *Solanum tuberosum* and *Solanum bulbocastanum*, wherein the plant comprises a segment of chromosome 8 of the *Solanum bulbocastanum* genome having a gene that confers the late blight resistance.

17. (New) The potato plant of claim 16, wherein the gene conferring the late blight resistance co-segregates with a marker selected from the group consisting of a $GO2_{586}$ RAPD

marker, a $PO9_{587}$ RAPD marker, a CT88 RFLP marker, a CT148 RFLP marker, a CT252 RFLP marker and a CT68 RFLP marker.

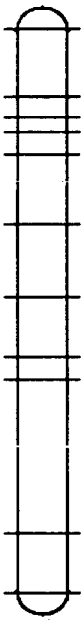
18. (New) The potato plant of claim 17 wherein the $GO2_{586}$ RAPD marker comprises SEQ ID NO:1, the $PO9_{587}$ RAPD marker comprises SEQ ID NO:2, and the CT88 RFLP marker comprises SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5.

19. (New) The potato plant of claim 16, which is also resistant to at least one additional disease selected from the group consisting of potato early blight, *Erwinia* soft rot, and *Verticillium* wilt.

EXHIBIT 1

Solanaceae Genomics Network

Stats for chromosome 8 of the Potato-TXB 1992 map.



Click on the chromosome to jump to the close-up map.

Length of mapped chromosome: 49.20 centimorgans

Markers:
This chromosome contains 19 markers.

Markers have the following distribution:

- F(LOD3) 0
- CF(LOD3) 0
- I(LOD2) 19
- I 0

Jump to a specific marker:
If you are looking for a specific marker, enter its name below to jump to a listing of its known map locations - both in this map and in all others registered with SGN.

Marker: CT88 Find marker

View abstract for the Potato-TXB 1992 map.

BACK to the Mapviewer top page.

This database is supported by the NSF (#9
and hosted at Cornell U

Send comments and feedback to sgn-feedback@sgn.cc

Solanaceae Genomics Network

Occurances of marker CT88

You came to this page from:				
Tomato-EXPEN 2000 map	Chromosome 8	offset 45.00	confidence F (LOD3)	Go there
Other mapped occurances of this marker are at:				
Tomato-EXHIR 1997 map	Chromosome 8	offset 37.1	confidence I(LOD2)	Go there
Tomato-EXPEN 1992 map	Chromosome 8	offset 47.1	confidence I(LOD2)	Go there
Eggplant-LXM 2002 map	Chromosome 8	offset 55.00	confidence CF (LOD3)	Go there
View SGN database page for CT88.				

List occurances for another marker:

Show Marker

Mapviewer main page

Search for multiply occurring markers.

This database is supported by the NSF (#9
and hosted at Cornell U

Send comments and feedback to sgn-feedback@sgn.cc

Solanaceae Genomics Network

Marker Retrieval

Marker ID CT88

Library Information No library information availabe

Forward Sequence Information

Source ID	CT88_M13F
Fasta Sequence	TTTTTTTTTTTTTTTTGTAGTTTAGAAGTGGCATAAAGTT GGGCCGAAGAGCTAGGAAGAGTAAGCATGTCAAGTGATAG TTGCAGCCACTGGTGTTATAGTTGTAGACAACCCGTGAAT CTCAGGAGACAAAATGATGTTTGCCCCAATTGCGGTGGTG GATTTGTTCAAGAGCTTGAAGACATAACGAGTAGTAGTGT AGATAATCAGACCCAGAGGCCGAGATTCATGGAATCCGTC TCAAACTTTTTAAGACGACAAATCTCAGCTACAAGTAATA CTTCTGAGAGAGGGAGATCTGATGGGGGTGCTGAACGAGG AAATTTGTGGAATCCGTTGCTGATTTTCAGTGGTGATACG CCTGTTCATATGCCTGGGGATGGTGGAGTTT

Reverse Sequence Information

Source ID	CT88_M13R
Fasta Sequence	TTCTGTTGTAGCCATGGCGTGATACAATCCGAGTGATATA AGTGCTTGCAAGGCAATTTTGTTGCCTTAGTCCCCAGAGC AAATTTCTCCTTACAAACAGGGCAGTGCGAATCCGATCTA ACATCCTTTTTTGATATCTTGACTGTTGGTAGGGAATCAA TTGAACATCTCGAGACAGGAGGAGGAGCACCACGCTGATT TCTATTTACAATTTCTTCAAAAAATTCCTCCACTCCTGGA CCAACAAAATAATCACCACCATTTTCTTGTCGGAATCCAA GTGCCTCATTAAGAAACTCCAAAACTCCACCATCCCCAGG CATATGAACAGGCGTATCACCACTGAAAATCAGCAACGGA TTCCACAAATTTCCTCGTTCAGCACCCCCATCAGATCTCC CTCTCTCAGAAGTATTACTTGTAGCTGAGATTTGTCGTCT TAA

Note: Sequences are derived from single pass sequence runs.
Note: X's in sequence are screened out vector sequence.

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Solanaceae Genomics Network

Occurances of marker CT148

Tomato-EXPEN 2000 map	Chromosome 8	offset 67.00	confidence F (LOD3)	Go there
Tomato-EXPEN 1992 map	Chromosome 8	offset 74.5	confidence I (LOD2)	Go there
Eggplant-LXM 2002 map	Chromosome 8	offset 78.00	confidence F (LOD3)	Go there
View SGN database page for CT148.				

List occurances for another marker:

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Solanaceae Genomics Network

Marker Retrieval

Marker ID CT148

Library Information No library information availabe

Forward Sequence Information

Source ID	CT148_M13F
Fasta Sequence	TCTCCAATTAAGCTTCGCGAAGAATCTACAAAAAAAGAGT CCTCCTTCAGCTCTTCATATGGAAACGCATTCTTCAAATC ACCAAACTTCCTCCGATAATGGAGTCGTTTTGCCTGAGTT GCTGACAGAGTTTATGGTGGATATGTCGTGTCAAGGCTGT GTTAGTGCTGTCAAGAGCAAATTGCAAACCGTAGAAGGAG TCAAGAATGTAGATGTGGATCTTGATAATCAAGTAGTGAG AATTCTTGGATCTTCACCTGTGAAGACAATGACTGAAGCC TTGGAGCAAACAGGTCGAAAAGCCCGTCTGATTGGGCAAG GAGTACCGGATGATTTCCTTATATCTGCTGCCGTTGCCGA ATTCAAAGGACCAGATATTTTTGGTGTTGTTCGCTTGGCT CAAGTCAATATGGAATTAACTAGGATTGAAGCAAACTTCA GTGGCCTGTCACCTGGGAACCATGCTTGGTCTATTAATGA ATTTGG

Reverse Sequence Information No available information

Note: Sequences are derived from single pass sequence runs.
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Solanaceae Genomics Network

Occurances of marker CT252

Tomato-EXPEN 2000 map	Chromosome 8	offset 84.00	confidence F (LOD3)	Go there
Tomato-EXPEN 1992 map	Chromosome 8	offset 89.1	confidence I (LOD2)	Go there
Eggplant-LXM 2002 map	Chromosome 8	offset 90.00	confidence F (LOD3)	Go there
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Solanaceae Genomics Network

Marker Retrieval

Marker ID CT252

Library Information No library information availabe

Forward Sequence Information

Source ID	CT252_M13F
Fasta Sequence	CTTTTTTTTTTTTTTGGTAATTTCAACTAAGAGTTAATAT ACTTCTTTCTTTTTTTTCTCTCTCTATAACTTCACAATTT TTTTGACTCTCTCTCTAAAAAGCTCAGAGATGAATTCTCA GATTTGCAGATCTGCTACAAGAGCAGCTAAGTCACTCCTT TCTGCTTCATCTAAGCAGACTTCTCGTGCTTTTTCAGGAG GACGAGCAGCAGCTGCAGCAGCCACAGTTTCATTGAGAGG AGTGGTGCCTTCTCTAGCCTCATATGGCAGGAATGAATCT GGAAATGCATCTAGAGCTTGGATTTCTGGTGTGCTTGCCC TTCCTGCAGCAGCTTACATGCTCCAGGAGCAAGAAGCACA TGCTGCCGAGATGGAGCGCACCTTTATTGCCATCAAGCCA GATGGAGTACAGAGAGGCCTGATTTCAGAAATCGTATCAC GGGTTGAGCGCAAGGGCTTCAAGCTGGTTGCAATCAAAGT TGTGATTCCTTCCAAGGAATTTG

Reverse Sequence Information No available information

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Solanaceae Genomics Network

Occurances of marker CT68

Tomato-EXPEN 2000 map	Chromosome 8	offset 87.00	confidence F (LOD3)	Go there
Tomato-EXPIMP 2001 map	Chromosome 8	offset 86	confidence F (LOD3)	Go there
Tomato-EXHIR 1997 map	Chromosome 8	offset 76.8	confidence I (LOD2)	Go there
Tomato-EXPEN 1992 map	Chromosome 8	offset 94.8	confidence I (LOD2)	Go there
Eggplant-LXM 2002 map	Chromosome 8	offset 101.00	confidence F (LOD3)	Go there
View SGN database page for CT68.				

List occurances for another marker:

Show Marker

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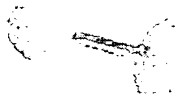

Solanaceae Genomics Network

Marker Retrieval

Marker ID CT68

Library Information

Library Name	CTpBS
Tissue	Tomato leaf epidermal
Accession	VFNT CHERRY
Description	Tomato leaf epidermal layer cloned into EcoRI site of pBluescript
Vector	pBluescript, Ampicillin resistance, M13F,M13R
Organism	Lycopersicon esculentum

Forward Sequence Information

Source ID	CT68_M13F
Fasta Sequence	CAGATCTACAGATGGAAGATCAATTGCAGACGATTGTCCG AGATCACTTGTAGAGCTGGAATCAATGTTACAAGCCAGAA AAGAAGCCTCCTTCAAGCGGGAAAAATCCCTTGCTCATGC TTTTACTCAACAGGAATTGGATGAGATGGATGCTGTTTGT AGTGAAGAAAGAAATGAAAGGGAATTAGAAGAGACAGCGA ATTGGCTAGACGAGTGGATGTCATCAAAGCAATGGAACAG AGGTTCATTTGACAGAACAGACTCTATAAAGACTGTTGAG AAGGACACGGCTAAGCCATATTGTAACATGGTTCTCAATG CTCTCAAGACACCACACTCTAGCCCACTTCGCAGACGGGC TAATAGTCCTCATTATACTGCTAATTCTCCCCATCACCAG AGATCATCACATTACGATTACTCGGCAATTCAACCACCAG CCACCCCACCCCCTTGTCAACCAGAACCTCTTCAAATGC

Reverse Sequence Information

Source ID	CT68_M13R
Fasta Sequence	CAGATCTAGAGATGGAAGCTCAATTGCAGACGATTGTCCG AGATCACTTGTAGAGCTGGAATCAATGTTACAAGCCAGAA AAGAAGCCTCCTTCAAGCGGGAAAAATCCCTTGCTCATGC TTTTACTCAACAGGAATTGGATGAGATGGATATTGTTTGT AGTGAAGAAAGAAATGAAAGGGAATTAGAAGAGACAGCGA ATTGGCTAGACGAGTGGATGTCATCAAAGCAATGGAACAG AGGTTCATTTGACAGAAGAGACTCTATAAAGACTGTTGAG ATGGACACGGCTAAGCCATATTGTAACATGGTTCCAAATG CTCGAAGATCACAACACTCTAGCCCACTTCACAGACAGGC TAGTAGTCCTCATTATACTGCTAATTCTCCCCATCACCAG AGATCATCACATTACAATTACTCGGCAATTCAACCACCAG CCACCCCACCCCCTTGTCAACCAAAACCTCTTCAAATGCG CTCAACAAGCCCACGTAAAAGCCAATCAACTGCAAACAC

Note: Sequences are derived from single pass sequence runs.
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EXHIBIT 2

USDA Plant Genome Research Program Progress Report

Anne Datko and Ed Kaleikau
National Research Initiative, CSREES, USDA
Steve Heller, Jerry Miksche and Garry Smith
Plant Genome Research Program, ARS, USDA
Doug Bigwood, Genome Informatics Group, NAL, ARS, USDA

The Plant Genome Research Program (PGRP) addresses agricultural problems. Solution of the problems will lead to efficient production of food, feed, and fiber while concurrently reducing the environmental impact associated with farming practices and manufacturing processes.

The goal of the program has remained the same for the past four years. It was established to speed the improvement of plants - agronomic, horticultural, and forest tree species. This is accomplished by locating and characterizing agriculturally important genes and by subsequent transfer of those genes to plants to improve performance. The products, through the hands of the breeder, will be new cultivars. These new cultivars will offer pest and disease resistance and tolerance to abiotic stresses, such as heat, drought, and cold. They will meet future marketplace needs and niches, as well as strengthen endeavors to enhance the environment.

The Plant Genome Research Program is a cooperative effort among several USDA agencies - the Agricultural Research Service (ARS) (which now includes the National Agricultural Library (NAL)) is the lead agency, and the Cooperative State Research, Education, and Extension Service (CSREES). ARS coordinates with the Human Genome Projects of the National Institutes of Health (NIH) and the Department of Energy (DOE), as well as the Arabidopsis thaliana effort of the National Science Foundation (NSF). The Plant Genome Research Program is in the process of establishing official cooperation with some of the European Union (EU) countries and informal but promising interactions with several Pacific Rim countries.

The USDA Plant Genome Research Program is a single program with two components:

- I. Competitive grants through CSREES, National Research Initiative.
- II. The Plant Genome Database through ARS.

The total appropriation from Congress for this program from 1991 through 1994 was $58.79 million. This amount represents $46.55 and $12.24 million dollars designated for the National Research Initiative competitive grants and the Agricultural Research Service, respectively.

I. COMPETITIVE GRANTS NRI COMPONENT

Grant proposals are oriented towards improving agronomic qualities through genomic research. The Request For Proposals (RFP) proposals addressing three categories: 1. Broad genome maps; 2. Fine maps, including physical mapping; and 3. Technology development to increase the efficiency of mapping and sequencing desirable genes.

Competitive grants were awarded to 381 scientists from 84 public, private, and government research institutions from 43 states. (Table 1). The average award per year over the four years was $127,177 with an average rate of success of 32.8% and an average of 2.2 years per award (Table 2). Fifty-one

agronomic, horticultural, and forest tree species and three non-agricultural taxa are included in the plant genome effort (Table 3). Eighty four percent of the grant funding went to members of five plant groups: 1. Tree species - $1.8 million; 2. Crucifers - $4.3 million; 3. Legumes - $5.9 million; and grasses - $16.6 million. Nearly 100 gene/trait/genetic phenomena are at various stages of progress as listed in Table 4. Table 5 lists some of the molecular biology technology pursued by the awardees of the technology over the past four years.

Several important accomplishments have been made by the Program grant awardees:

- For the first time ever, a disease resistance gene was isolated by map-based cloning technology. The bacterial speck resistance gene was molecularly transferred to a susceptible variety, resulting in resistance.

- One awardee is part of a team that discovered a new class of genes that allows a plant to recognize a diverse group of pathogens.

- Quantitative trait loci (QTL) methods have been used to develop a barley line resistant to barley stripe rust which increased the yield of corn by 15%.

- Researchers have analyzed the loblolly pine genome and mapped over 200 genetic markers as part of a tree improvement program in the Southeastern United States. Tree breeders can now expedite the improvement of loblolly pines by shortening the time necessary to select desirable characteristics and by using these genetic markers to identify trees with desirable traits.

The above achievements represent only a small fraction of the program's effort. Development of molecular genomic maps for corn, wheat, sorghum, soybean, cotton, tomato, peanut, lettuce, apple, and other commodities is progressing rapidly and the association of the markers with desirable genetic traits will facilitate plant breeding and crop improvement efforts. This activity of placing the DNA gene locations in the hands of the breeder is a paramount goal of the USDA Plant Genome Research Program.

II. PLANT GENOME DATABASE COMPONENT

With the aid of molecular geneticists and breeders, the PGRP is achieving its aims by locating and using genes that improve plants. The program uses the intellectual prowessof government, university, and private sector researchers.

Collaboration of many researchers throughout the country and the world necessitates the need for electronic media communication. Plant genome research generates voluminous data and the handling of large amounts of information requires researchers to have computer expertise.

Performing experiments in the laboratory or field and publishing results in regular scientific journals will not adequately meet the information demands of the 21st century. The ability to rapidly assimilate, analyze, and compare research findings in an electronic form will be needed.

Voluminous data generation requires a Plant Genome Database (PGD). It is divided into three components:

1. Stock Center Databases - These databases consist of data necessary to enhance genomic research. They are located throughout the United States and elsewhere and are of utmost importance to the USDA PGD and plant breeders in general to making the desired genetic variation available to

address agricultural problems. At present, there is a database called GRIN, Germplasm Research Information Network, and while not a direct part of the plant genome research program, it provides a valuable link between germplasm and the genetic information from the other databases (See "Germplasm Resources Information Network (GRIN)" in this issue).

2. Genome Mapping Database includes physical, genetic, RAPDs, RFLPs, and other map types of many agricultural plant species and some model systems from non-agricultural taxa. These databases are being developed for the first time in a coordinated manner under the direction of the program. At present, ARS is funding directly and indirectly the databases for Arabidopsis thaliana and Chlamydomonas species (model systems), apple, barley, beans, corn, cotton, oats, peas, pepper, petunia, pine, poplar, potato, rye, sorghum, sugarcane, tobacco, tomato, Triticum species -- and the list is expanding.

3. DNA Sequences - Since this data is the same as those which are being placed into GenBank/GenInfo/European Molecular Biology Laboratories (EMBL) and DNA Databank of Japan (DDBJ), it was the consensus not to have a separate independent sequence database. Hence, all plant DNA sequence data from the USDA Plant Genome Research Program are being placed in the above sequence database efforts of the Plant Genome Database program.

The Agricultural Research Service is concerned only with mapping databases through a rather simple but efficient process. Funding, direct and indirect, is supplied by ARS to "database curators" for the various species. These researchers take the lead for their respective species, perform some evaluation and quality control of their databases, and hold that information in the home laboratories of their institution. The information is sent to the USDA National Agricultural Library, where it is integrated into a master or central database system with data from all of the species. The priority database topics include disease/ pathology, genetic resources, germplasm, genetic maps, quantitative traits, and other factors as decided upon by the database operators. Links are made to relevant databases such as GenBank/EMBL/ DDJB, SwissProt, and AGRICOLA.

All of the information in the database is accessible to the public. The Plant Genome Database is now a real and functioning information and data resource for agricultural genome researchers. As the system increases in size and intellectual content, its value will greatly increase and enhance the abilities of researchers to undertake more sophisticated genome research, which will ultimately benefit the agricultural community and all consumers.

EXHIBIT 3

Baker Lab

Isolation of *Phytophthora infestans* Resistance Genes from the Mexican Wild Species Solanum demissum

Research

N gene

yeast 2 hybrid

acitvation tagging

bio-chem

late blight

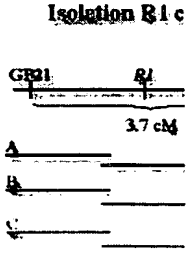
VIGS

The Late blight disease of potato, by the oomycete pathogen Phytop infestans (P. infestans), is one of the devastating of all plant diseases. *P. in* causes annual losses of up to 5 billion worldwide with increasing losses sin spread of fungicide-resistant Phytophtho are isolating *P. infestans* resistant (R) from wild species Solanum demiss provide a foundation for the developm novel, durable broad-spectrum resistanc

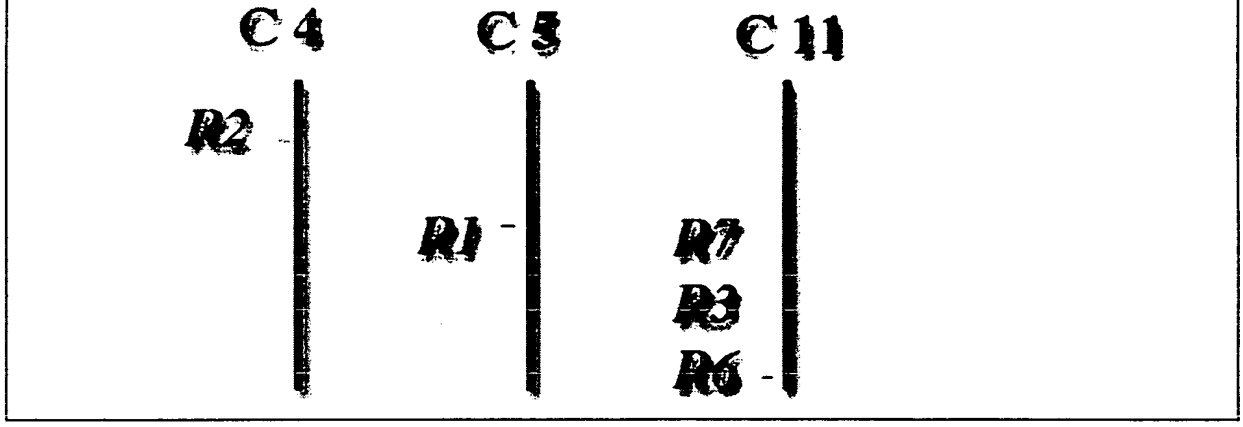
S. demissum has been shown to field resistance genes and contains at le race-specific R genes (R1-R11) through hexaploid genome. Resistance mediat R1-R11 genes is associated wi hypersensitive response. Our lab will fo the isolation and characterization t *infestans R genes, R1 and R7.*

Positional cloning of potato late blight disease resistant genes

R genes are generally found clustered. It is known that the late blight resistant gene R1 region has other disease resistant genes, including Rx2 and Nb genes for potato PVX virus resistance, and potato cyst nematode resistant genes. The late blight resistant R7 gene region also harbors late blight race specific resistant gene R3 and R6. We anticipate that from this work, not only that late blight resistant genes will be cloned, but also that sequences from the BAC contig will facilitate isolation of other disease resistant genes. More interestingly, sequences of the 3 solanum demissum genomes will provide rich information on genomic organization of R genes and molecular mechanism of R gene evolution.



Mapped *P. infestans* R genes from *S. demissum*



1 2 3 4 5 6 7 8 10 Next 10

EXHIBIT 4

Agricultural Research Service

TEKTRAN

CHARACTERIZATION AND MAPPING OF RPIL, A LATE BLIGHT RESISTANCE LOCUS FROM DIPLOID (1EBN) MEXICAN SOLANUM PINNATISECTUM

Author(s):
KUHL JOSEPH C
HANNEMAN JR ROBERT E
HAVEY MICHAEL J

Interpretive Summary:
Wild Solanum species grow naturally from the southwestern United States to central Chile and show many resistances to economically important disease of the cultivated potato. Research has focused on wild species that easily cross with the cultivated potato. Wild Mexican Solanum species have received less attention because of poor crossability with cultivated potato. Late blight is a devastating fungal disease of potato caused by Phytophthora infestans. We characterized resistance to P. infestans in the Mexican species Solanum pinnatisectum. A hybrid between resistant S. pinnatisectum and susceptible S. cardiophyllum plants was backcrossed to S. cardiophyllum to generate a family segregating for late blight resistance. A genetic map generated with 99 differences in the DNA and revealed a single dominant resistance gene, named Rpi1, on chromosome 7. This chromosome region has not been associated with late blight resistance, indicating that this could be a new resistance gene. These results will be of interest to potato breeders and pathologists who can use this gene as a new source of resistance to late blight.

Keywords:
vegetables plant breeding flavor quality rflp onion garlic carotenes nutritional value tissue culture disease resistance linkage map genetic markers cucumbers melon carrot

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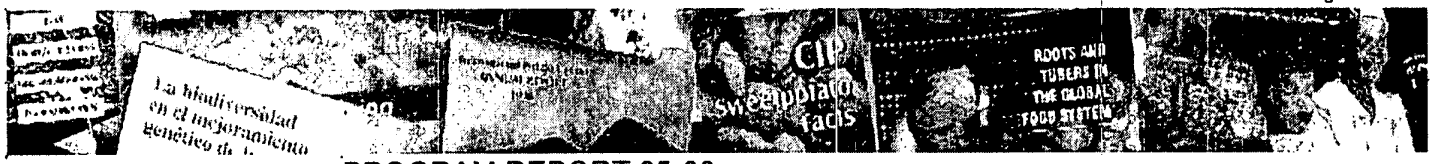
Approved Date: 2000-12-04

TEKTRAN
United States Department of Agriculture
Agricultural Research Service

Updated: 2001-01-24

EXHIBIT 5



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- Program 4: Integrated

Program 3

Evaluation of Late Blight Resistance in Populations of Diploid Potato Hybrids for Genetic Mapping

B. Trognitz, M. Ghislain, G. Forbes, P. Oyarzún, M. Eslava, R. Herrera, L. Portal, P. Ramón, and G. Chacón[1]

Wilde and cultivated relatives of the potato (*Solanum* spp.) carry valuable resistances to late blight (LB) that, when introgressed into potato, are thought to considerably reduce the crop's vulnerability to this devastating disease.

To increase the durability of resistance to LB, researchers seek forms of resistance that are effective against a broad range of pathogenic strains of *Phytophthora infestans*. Polygenic, additive resistance is the most promising. This resistance can be masked by monogenic, race-specific major

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- CIP Staff in 1995-1996
- Acronyms and Abbreviations

(R) gene-mediated resistance when no races compatible with such an R gene are available. This is most important when the identity of R genes is not known.

Twelve R genes for resistance to LB have been identified. Differential potato clones to distinguish 11 of them are available. These 11 R genes originated in Mexican wild *S. demissum*, and this and other Central American potato relatives are assumed to have developed many more R genes. The Mexican wild potato *S. verrucosum* is reported to possess high levels of quantitative resistance besides R gene-mediated resistance. The successful use in resistance breeding of this and other wild and native cultivated potatoes therefore depends on knowledge of the occurrence and identity of R genes.

One strategy to avoid interference with R gene resistance could be to choose genotypes without R genes. Previously, it was hypothesized that potato relatives indigenous to South America would not possess R genes; all resistance expressed by them would be polygenic and quantitative. However, evidence is emerging that many South American species also employ R genes. Therefore, it is desirable to test every potential source of resistance for the occurrence of race-specific resistance genes, and it is reasonable to expect that additional R genes are yet to be identified.

Two sources of resistance to LB were investigated in two segregating populations of diploid potato hybrids. One population was a cross between highly resistant *S. verrucosum* and susceptible *S. phureja*, designated population VP. The other was a cross between resistant *S. phureja* and a susceptible *S. tuberosum* dihaploid, designated population PD. The PD population was selected for the study from nine diploid hybrid populations carrying high levels of resistance to LB.

This investigation had two objectives. One was to characterize the level of resistance of every individual of the two populations as a precondition for genetic mapping. The second was to test the populations for the occurrence and segregation of race-specific R genes.

Population VP

This population comprises 102 individuals, all of them late-maturing under the short daylength of Cajamarca, Peru. All genotypes form small, pear-shaped tubers of creamy-white flesh that sprout early. The entire population expresses a cytoplasmic male sterility (CMS) phenotype that is known as eclipse sterility. Although preliminary intrapopulation crosses were unsuccessful, it may be possible to use the VP individuals as females in crosses with pollen-fertile genotypes because female fertility is not affected by CMS.

VP field resistance

VP individuals were evaluated for resistance in the field at Cajamarca, Peru, under high infection pressure, in 1994, 1995, and 1996. The parents did not grow in the field or were not available and

could not be included in the experiment. A randomized block design was used and the area under the disease progress curve (AUDPC) was calculated from weekly readings of the percentage of diseased foliage in plots of 10 plants per clone and block. The VP population had a high average field resistance (progeny mean, AUDPC=303, range 168–999; resistant standard Perricholi, AUDPC=410; susceptible standard Yungay, AUDPC=997). Its frequency distribution (Figure 1) deviated from the desired normal distribution typical for a quantitative trait.

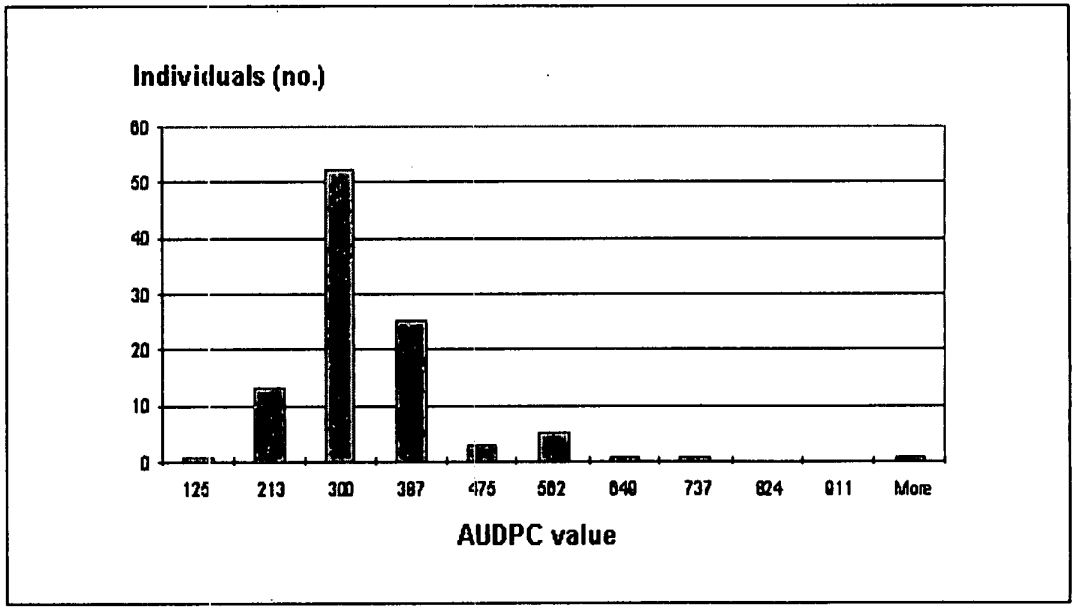

Figure 1. Population VP: frequency distribution of AUDPC measured on 99 individuals in 3-yr field trial, Cajamarca, Peru.

Race-specific resistance of VP

We performed detached-leaflet tests to analyze the segregation of the discrete trait resistance to sporulation. The phenotype of R gene resistance in this test is a hypersensitive response of leaf tissue. Sometimes no symptoms or only weak infection is observed. Sporulation occurs rarely. We classified individuals as susceptible or resistant based on this variability of the expression of resistance. Individuals on which *P. infestans* sporulated were classified as susceptible; those that dic not allow the pathogen to sporulate in any of the repetitions were considered resistant.

Ninety-nine individuals were inoculated with five isolates of *P. infestans*, each possessing a different level of virulence (Table 1). The experimental unit was a petri dish containing four lateral leaflets from different fully developed top leaves of one to three plants in bud or flowering. The plants were grown in pots in a greenhouse at Lima during the winter of 1995. Humidity was maintained by adding a sheet of moist filter paper to each petri dish. Readings of sporulation and area affected were taken 5 d after inoculation, or when the controls showed the expected symptoms. Tests were repeated one to three times and the accuracy of a test result was established by comparing it with the reaction of resistant and susceptible control cultivars.

Table 1. Inoculations of detached leaflets of VP individuals with 5 isolates of *Phytophthora infestans*.

Isolate	Virulence	Resistant progenies (no.)	Susceptible progenies (no.)	Segregation observed	Segregation expected for single-gen resistance
275	1	36	63	1:1.75	1:1
P2	1.3.4.6.7.10.11	30	69	1:2.3	1:3
50	1.3.4.7.11	15	84	1:5.6	1:3
8	1	0	99	0:1	1:1
260	1.2.3.4.6.7.9.10.11[a]	0	99	0:1	0:1

a. Not tested against R8.

The inoculum was applied with a spray bottle. The inoculum concentration was 5,000–15,000 sporangia/ml washed from mycelium grown on tuber slices. This high inoculum concentration was chosen to ensure that all susceptible individuals became infected.

Of 99 plants tested, none was resistant to isolates 8 and 260. With the remaining three races, segregation into resistants and susceptibles was obtained (Table 1). The ratios of resistant:susceptible individuals obtained with either race significantly ($P<0.001$) diverge from ratios expected for a model of single dominant genes of resistance. More feasible models were the complementary action of two or more resistance genes, or a resistance gene and a suppressor

gene. In dozens of inoculations done over 5 yr, our controls carrying R genes always responded resistant to the respective avirulent isolates. None developed sporulating mycelium. Possibly the resistance genes of *S. verrucosum* break down under particular environments, thus resulting in an excess of susceptibles. Segregation results must be confirmed by testing the V and P parents and by analyzing backcrosses to the susceptible *S. phureja* parent.

A host-pathogen interaction scheme is presented in Table 2. The eight resistance patterns observed in the population indicate that the virulences of the isolates 275, P2, and 50 are different from each other. At least three factors of race-specific resistance must be assumed to segregate independently in the VP population. Isolates 8 and 275 share the avr1 gene for virulence on potato (*S. demissum*) gene R1 (Table 1), yet they have differing patterns of compatibility with the VP individuals. That result indicates that isolate 8 possesses more virulences either to R gene 8 (for which no differential was available) or to some other unknown resistance genes.

Table 2. Response[a] of 99 VP plants to inoculation of detached leaflets with five isolates of *Phytophthora infestans*.

Isolate					Individuals resistant (no.)
275	P2	50	8	260	
S	S	S	S	S	43 susceptible
R	S	S	S	S	20
S	R	S	S	S	13
S	S	R	S	S	4
R	R	S	S	S	8
S	R	R	S	S	3
R	S	R	S	S	2
R	R	R	S	S	6
a.	R = resistant to development of sporulating mycelium, S = susceptible.				

After 3 yr of resistance testing in the field, the AUDPC means of genotypes differing in their resistance to 0, 1, 2, or 3 isolates were compared by a series of t-tests (Figure 2). None of the

means could be clearly separated. But the tendency of smaller AUDPC values to be associated with a higher number of isolate-specific resistances indicates a small but favorable residual effect of these factors on the expression of resistance in the field. Also, the mean AUDPC value (mean AUDPC=280) of all 36 individuals that were resistant to isolate 275 was smaller than that of the individuals susceptible to that isolate (mean AUDPC=317, 63 individuals).

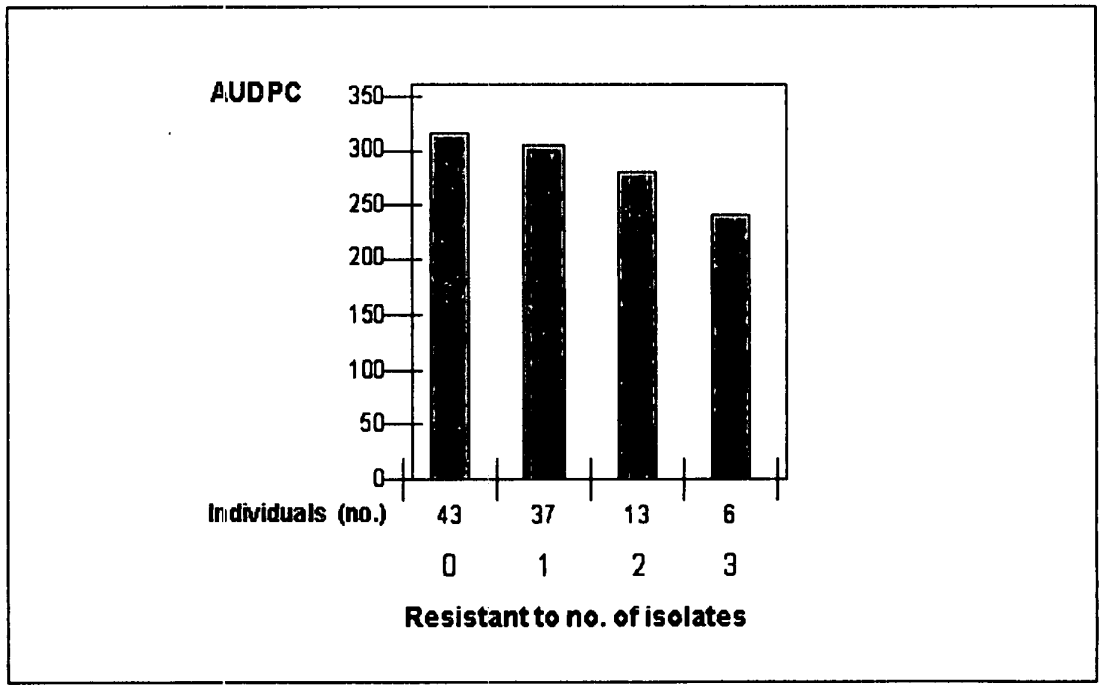

Figure 2. Mean AUDPC values of classes of VP individuals resistant to 0-3 isolates.

Population PD

PD is the result of a seedling family selection process. A sample of accessions of wild and native cultivated potatoes as candidate sources of resistance to LB was screened at CIP-Quito, in 1992 and 1993. Resistant clonal selections were crossed with potato dihaploids to produce diploid hybrid

progenies. A sample of nine early-maturing progenies was subjected to a 2-yr field trial for resistance. The cross CHS-625 x PS-3 performed best of all progenies. Its progeny mean of resistance (AUDPC=152, 50 individuals) was, of all nine progenies, closest to the value of the resistant standard, variety Catalina (AUDPC=98). The individuals had AUDPC values between 65 and 240, displaying a normal distribution. Twenty percent of the individuals had the same resistance as the standard, or a higher level. All individuals of this progeny are male- and female-fertile and 65% of them produce more than 2% unreduced pollen grains. This progeny also has smooth round and oval-shaped tubers with shallow eyes and yellow skin. The yellow-fleshed starchy tubers have good culinary quality. The cross of the parents was repeated in Peru to produce the PD mapping population.

All individuals of population PD are male- and female-fertile and flower profusely in the greenhouse at CIP-Huancayo, Peru. Plants are vigorous, but seem to be vulnerable to infection by mosaic viruses—a feature frequently observed in wild and native potatoes. Introgression of virus resistance in subsequent crossing generations will be necessary.

PD resistance in a controlled-environment test

PD plants and their parents were grown in pots in the greenhouse at Lima, during the 1996 winter season. Stems with complete foliage were used in a resistance trial in a screenhouse equipped with mist irrigation to constantly maintain high relative humidity and low temperature. A randomized 2-block design was used. The experimental unit was a milk bottle containing three stems of a PD genotype. The plants were inoculated at night with isolate 260 (complex virulence to S. demissum R genes, see Table 1), at a concentration of 13,000 sporangia/ml. The epidemic developed after 4-7 d and visual readings of the percentage of diseased foliage were taken three times at 2-d intervals. The experiment was repeated after 4 wk, and a two-factor ANOVA (factors PD genotype and block, nested in repetition) was run on the mean percentage of diseased foliage calculated over the three readings. There were significant differences between the PD individuals, although no groups could be separated by multiple comparisons of means. Based on the conditions of the screening facility, the overall level of disease was different for each block within each repetition.

Figure 3 shows the frequency distribution of diseased foliage for the PD individuals, which fits a normal distribution. The overall level of resistance of the PD population (27% diseased foliage) observed was higher than that of the resistant control, Canchán (4%). But it was much lower than that of the susceptible check, Yungay (33%).

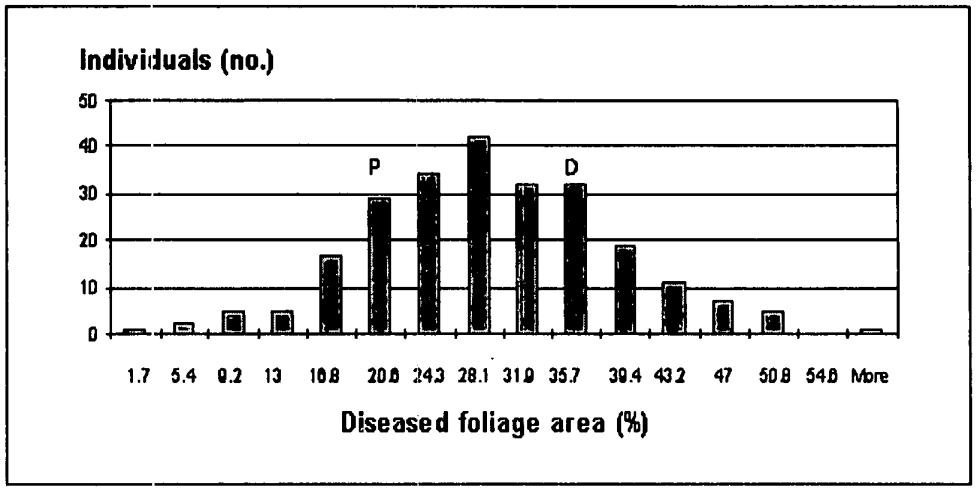

Figure 3. Population PD: frequency distribution of diseased foliage area (%) measured on 240 individuals and the P and D parents in 2 repetitions, Lima, 1996.

Overall, high levels of resistance that vary gradually between sister individuals depict the image of true quantitative resistance caused by many additive genes. Thus, the PD population is useful for molecular mapping of quantitative trait loci for resistance. More resistance studies must be done under actual field conditions and in different environments to elucidate the stability and sustainability of the resistance detected.

Testing for R genes in population PD

Most of the PD individuals developed foliage damage of more than 15-20% after inoculation with a virulent isolate of *P. infestans* (Figure 3). A small fraction of individuals exhibited less than 10% damage and preponderantly necrotic lesions. Therefore, it is preferable to test this population for the segregation of putative, isolate-specific resistance as well.

Two repetitions of a test for sporulation on detached leaflets of 218 PD individuals were done using the isolate P2 (Table 1). Of 218 individuals tested, 9 did not sporulate in one of the two repetitions, and 13 did not sporulate at all. No necrotic lesions developed, and the size of the lesions that did develop on these nonsporulating leaflets was similar to that on leaflets of other individuals on which the pathogen sporulated. Further tests with this and other isolates of *P. infestans* on the PD individuals and on progenies from crosses of sisters and backcrosses to the D parent are necessary to elucidate whether race-specific resistance is also segregating in this quantitatively LB-resistant material.

Conclusions

Population VP expressed high levels of resistance in the field, with little variation among genotypes. Besides the population's overall field resistance, race-specific interaction of individuals was detected in detached-leaflet assays. These race-specific resistances had a small favorable effect on resistance in the field.

We will use male-sterile individuals of the VP population as females in backcrosses with the susceptible P parent and with a dihaploid tester clone to obtain advanced backcross populations for further analysis of the resistance and use in breeding.

Population PD had a wide range of gradually different resistance levels in a repeated controlled-environment, foliage resistance assay, thus allowing the separation of most- resistant from most-susceptible individuals by statistical means. High levels of quantitative resistance to LB as well as good fertility and the production of unreduced (2x) gametes, earliness, yellow tuber flesh, and other good agronomic characteristics of the PD population make it a valuable material for breeding. Introgression of resistance to LB from this population into cultivated potato appears to be possible. To carry out this introgression efficiently, we will need to genetically map quantitative resistance loci as a precondition to marker-assisted selection.

Selected Reading

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